

Standard Operating Procedure for:
LS 13 320 Laser Diffraction Particle Size Analyzer Operation
(Particle Sizer R01.doc)

Missouri State University
and
Ozarks Environmental and Water
Resources Institute (OEWRi)

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1 Identification of the method

Operation of the LS 13 320 Laser Diffraction Particle Size Analyzer.

2 Applicable matrix or matrices

This instrument can be used for natural sediment samples.

3 Detection Limit

The aqueous liquid module (ALM) is capable of suspending samples in the size range of 0.04 μm to 2000 μm . The Polarization Intensity Differential Scattering (PIDS) assembly provides the primary size information for particles in the 0.04 μm to 0.4 μm range. The PIDS assembly also enhances the resolution of the particle size distributions up to 0.8 μm . This additional measurement is necessary as it is very difficult to distinguish particles of different sizes by diffraction patterns alone when the particles are smaller than 0.4 μm in diameter. Repeatability is 1% about mean size.

4 Scope of the method

This standard operating procedure provides Missouri State University (MSU) Ozarks Environmental and Water Resources Institute (OEWRI) laboratory personnel procedures for the operation of the LS 13 320 Laser Diffraction Particle Size Analyzer.

5 Summary of method

Sediment samples are collected and stored in ambient conditions in Temple Hall. Information about the sample (i.e., collection time, temperature, preservative, etc.) is recorded on the sample bag and sample collection form. Sediment samples are disaggregated, sieved to < 2 mm size, and transferred to sample tubes. Samples are pretreated with hydrogen peroxide and acetic acid to remove organic matter. The addition of sodium-hexametaphosphate enhances separation and dispersion of aggregates before sonification. The samples are loaded into the auto-prep station (APS) after pretreatment.

The APS sonicates each sample and adds the sample to the ALM bath by flushing the sample tube with water. The ALM contains filtered tap water that suspends the sample and the ALM sonicates the sample and uses a circulating pump to disperse the sample in the tap water. Water used for this analysis is pumped from a holding tank that contains heaters which keep the water at a constant temperature. The sample is re-circulated in a closed-loop system while it is delivered to the sample cell in the optical bench.

The optical system (Figure 1) consists of a source of illumination, a sample chamber where the sample interacts with the illuminating beam, a Fourier lens system that focuses the scattered light, and an array of 126 photodetectors that record the scattered light intensity patterns. Diffraction scattering patterns from 0.4 μm to 2000 μm are measured by 119 of the 126 photodetectors. The remaining seven detectors are associated with the PIDS assembly and measure particle size in the 0.4 μm to 0.4 μm range.

When a sample reaches the sample cell, the sample load is measured and diluted until a standard obscuration percentage is attained. Then the detector array records the composite diffraction scattering pattern of the sample. The size distribution is computed by straightening the set of numbers for each size classification which are represented by each channel detector. The relative amplitude of each number is used

as a measure of the relative volume of equivalent spherical particles of that size class. The straightening of the data set is based on the Fraunhofer or Mie theories of light scattering from particles.

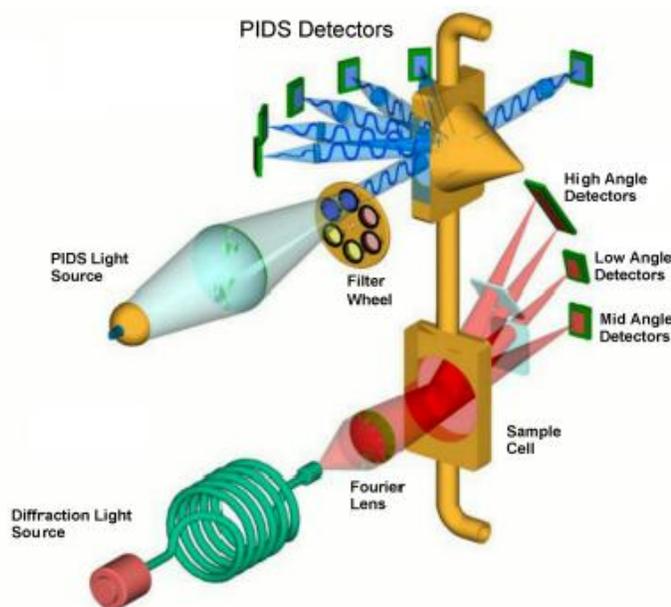


Figure 1. LS 13320 optical system

It is difficult to distinguish particles of different sizes by diffraction patterns alone when the particles are smaller than $0.4 \mu\text{m}$ in diameter. Therefore particles in the $0.04 \mu\text{m}$ to $0.4 \mu\text{m}$ range are measured by the PIDs assembly that utilizes the remaining 7 of 126 photodetectors in the array. The PIDS assembly illuminates the particles sequentially with vertically and horizontally polarized light from three different visible wavelengths and the differential scattering patterns produced are measured 36 times. One photodetector is dedicated to measuring the intensity of the un-scattered light to determine the amount of obscuration. The PIDS measurements are added to the same straightening matrix that is used for determining size distribution using laser diffraction patterns alone to integrate the two measurements methods and produce one data set.

6 Definitions

- 6.1 Alignment Measurement: LS 13 320 pre-run cycle procedure that monitors the alignment of the laser beam to ensure that it is automatically aligned within 1-2 microns of the center of the detector array on the horizontal and vertical axes. This procedure obtains the light intensity patterns (diffraction or PIDS) from which the size distribution is calculated and ensures instrument accuracy.
- 6.2 Analytical batch: A set of samples processed at the same time.
- 6.3 Aqueous Liquid Module (ALM): The Aqueous Liquid Module presents the entire sample to the sample cell in the optical bench by re-circulating the sample through hoses. The system is controlled using software commands. The ALM system consists of: the sample vessel containing the suspension fluid and dispersed

sample particles; the sample cell; the sonicator that aids in the dispersion of the sample during the run cycle; the variable speed circulation pump that circulates the particles through the sample cell; the inlet and drain hoses; the hoses to transfer fluid from the vessel to the sample cell; and liquid level sensors.

- 6.4 Auto Prep Station (APS): The Auto Prep Station is used in conjunction with the LS 13 320 optical bench and the Aqueous Liquid Module (ALM). It is capable of adding dispersant as well as sonicating a total of thirty aqueous samples. The APS transfers the entire amount of each individual sample to the ALM vessel by rinsing the sample tube with water for a preset, OEWRI-selected time.
- 6.5 Background: The background flux pattern provides information about the cleanliness and integrity of the tap water used for sample dilution. The background flux intensity limits are as follows:
- The flux counts within Channels 1 and 2 must be $<4.0 \times 10^6$.
 - The flux counts for the sum of Channels 10 through 40 must be $<2.0 \times 10^6$.
 - The Channel between 95 and 126 that has the highest flux counts cannot exceed 30.
- 6.6 Bench sheet: The sample tracking sheet used in the laboratory that links sample identification and sample procedures performed. The bench sheet for these procedures is located in Appendix A.
- 6.7 Calibration verification standard (CVS): A standard consisting of a known sample media used to calibrate the instrument during initial setup, monitor the instruments recognition of initial calibration, and to quantify batch accuracy. Results of CVS analyses are reported as % differential volume. CVS used for this procedure are provided by the instrument manufacturer (Beckman Coulter) and consist of two size fractions: 15 μm garnet (G15) and 500 μm glass beads (GB500).
- 6.8 d10, d50, d90: Particle size distribution is reported as the grain diameter at 10% (fine tail), 50% (median), and 90% (course tail) of a cumulative percent frequency distribution curve where each point illustrates the percentage of the total sample weight accounting for the weight of all grains smaller than a certain diameter (d). This allows the description of wide ranging data sets, since particle diameters can span many orders of magnitude for natural sediments. The LS 13 320 actually accounts for the area of the cumulative curve and not the weight because the instrument dilutes the sample to decrease interference during scattering.
- 6.9 Detector channel: Various optical paths, or focus areas, of a detector array associated with laser diffraction. Each channel consists of a light sensitive detector surface and associated amplifying electronics.
- 6.10 Field duplicate (FD): Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory

procedures.

- 6.11 Laboratory duplicate (LD): An aliquot of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.
- 6.12 Laser diffraction: Technology that correlates laser beam diffraction patterns to particle size. Particles are suspended in water, diluted, and passed through a cell where a laser beam is directed. Particles scatter the beam and a series of photodetectors placed at different angles measures the diffraction pattern caused by the scattering. Smaller particles scatter the laser beam at larger angles than bigger particles. Light scattering theory is used to derive the sizes of particles in the sample and particles are assumed to be spherical. Samples are diluted to omit interference by re-scattering.
- 6.13 Measure Loading: A LS 13 320 function that measures the amount of light scatter from the presence of particles from each sample, calculates loading rates, determines the prescribed obscuration percent as specified in the Standard Operating Method (SOM), and dilutes the sample until the specified obscuration percentage is obtained. Sample dilution deletes interference between particles within the sample which sharpens the light intensity pattern and provides an acceptable signal-to-noise level in the detector channels.
- 6.14 Minimum Quantification Interval: The lowest level that can be quantified accurately and is generally defined as four times the method detection limit = 4(MDL).
- 6.15 Natural standard (NS): A sample of a known particle size distribution and that has composition similar to environmental samples being analyzed. The NS undergoes the same preparatory and determinative procedures as the environmental samples and is used to evaluate accuracy along with the calibration verification sample (CVS). ES63 and ES250 are used for OEWRI projects. Both originate from an engineering sand sieved to produce a size range of $63\mu\text{m} < x < 125\mu\text{m}$ and $250\mu\text{m} < x < 500\mu\text{m}$ respectively.
- 6.16 Obscuration: The amount of light scatter from the presence of particles within a laser beam. Run cycle options within the Standard Operating Method (SOM) have been preset to produce desired obscuration values for laser diffraction alone or with the use of polarization intensity differential scattering (PIDS) analysis. Project management and laboratory personnel should discuss the anticipated fractions of the environmental samples prior to analysis to determine if the additional PIDS enhancement is desired which will dictate the obscuration setting.
 - a. Appropriate obscuration values for analyses using laser diffraction alone are 8% to 12%.
 - b. When PIDS sizing is used in addition to laser diffraction, the obscuration should be set to *Standard Obscuration* in the

corresponding SOM. This setting will allow sample analysis when PIDS values range from 40 to 60%.

- 6.17 Offsets: Bias voltages from the amplifier circuit of the instrument that may cause electrical noise in a detector channel. These bias voltages from the amplifier circuit are measured before every run cycle in order to “zero out” or establish an electrical noise baseline at the detector channel. The signal at each detector channel does not go to zero in the absence of light, so the measured bias voltage intensity is subtracted from the diffraction pattern from the sample to improve sizing accuracy.
- 6.18 Polarization Intensity Differential Scattering (PIDS): An assembly that provides the primary size information for particles in the 0.04 µm to 0.4 µm range and enhances the resolution of the particle size distributions up to 0.8 µm. This is an additional measurement that differentiates particles smaller than 0.4 µm in diameter that are indistinguishable using laser diffraction patterns alone.
- 6.19 Preference file (.prf): A program that includes options relating to data presentation and output formats. The preference file used in this procedure is: OEWRI_Standard_Preferences.prf. This preference file exports grain size distribution data for each sample as differential volume % for each of the 126 channel listings. Statistical data consisting of mean, mode, d10, d50, and d90 is exported for each sample with the preference file as well. Project specific reference files can be created or edited to fit project specific needs as determined by the project manager and laboratory director.
- 6.20 Relative Percent Difference (RPD): A measure of method precision calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

$$\text{Relative percent difference (RPD): } \left[\frac{(A-B)}{\text{AVERAGE}(A,B)} \right] 100$$

A = original sample concentration
B = duplicate sample concentration

- 6.21 Standard Operating Method (SOM): Programs that control the instrument settings and automatic sampler to ensure consistent analyses and improve precision. The SOM that is used for natural sediment samples is titled OEWRI_Standard_SOM.som and includes preset sample analysis protocol pertaining to run cycle options, Auto Prep Station sample handling, and run settings. Project specific SOMs can be created or edited to fit project specific needs as determined by the project manager and laboratory director.
- 6.22 Standard Operating Procedure (SOP): Protocol that consists of instrument and Auto Prep Station settings as well as data presentation and data output formats. The SOP is divided into two distinct components; a SOM (Standard Operating Method) (.som) file and a Preference (.prf) file.

7 Interferences

External mechanical vibration, if present, may cause misalignment of the laser. Automatic alignment occurs before every analysis as preset into the SOM. Electrical interferences can occur, but are unlikely because the instrument is connected to an uninterruptible power supply. Sudden changes in temperature can cause misalignment as well as changes to the measured electrical offsets. Users will record the time and temperature at the instrument before analysis. Optimal temperature range is 10 to 35°C and relative humidity range is 0 to 85%. The optical bench is completely enclosed so that background laboratory light does not interfere with scatter patterns. The Fourier lens focuses on the incident beam so it will not interfere with the scattered light. The instrument is calibrated by the manufacturer and certified installer.

8 Health and safety

The LS 13 320 contains a 5 mW diode laser. This Class 3a laser does not produce a hazard if viewed for only momentary periods with the unaided eye, but may present a hazard if viewed using collecting optics. The instrument therefore may pose certain hazards associated with low-power lasers if misused. All maintenance procedures associated with the laser will be performed by the laboratory supervisor only. Never look directly into the laser light source or at scattered laser light from any reflective surface. To comply with Federal Regulations (21CFR Subchapter J) as administered by the Food and Drug Administration's (FDA) Center for Devices and Radiological Health (CDRH), defeatable microswitches are located on the right and left of the door panel. Do not tamper with or attempt to defeat the safety interlock switches. A copy of ANSI standard 2136.1, SAFE USE OF LASERS, is located near the instrument for reference.

Review the MSDS for information and safety concerns regarding the standard powders or dispersants used throughout these procedures. Specifically note that a 30% hydrogen peroxide (H₂O₂) solution is used during sample pretreatment and should not come in contact with the skin because it is a strong oxidizing agent.

9 Personnel qualifications

Samples will be analyzed by Missouri State University (MSU) Ozarks Environmental and Water Resources Institute (OEWRI) laboratory personnel who have received appropriate training from experienced personnel, prior coursework, and laboratory experience regarding the analyses, and who are familiar with all of OEWRI's sample handling and labeling procedures and appropriate SOPs.

10 Equipment and supplies

10.1 Beakers: 10ml and 250ml

10.2 Carousel: part of Auto Prep Station assembly; loads samples into the instrument, 30 sample capacity.

10.3 Centrifuge: International Clinical Centrifuge, 1.2 amp, serial # AC1428.

- 10.4 Chain of Custody Forms: used to describe the written record of the collection, possession and handling of samples. Chain of custody (COC) forms are located on a board in Temple Hall 125. Chain of custody forms should be completed as described in the Chain of Custody SOP # 1030R01.
- 10.5 Daigger Vortex Genie 2: Tube votex, cat no. G22220.
- 10.6 Flasks: 500ml and 1000ml
- 10.7 Labeled sample tubes: 13ml
- 10.8 Micro-spatulas: used to transfer sample or standards into sample tubes.
- 10.9 Protective Gloves: for protection against chemicals and from contaminants.
- 10.10 Sonicator: Omni Ruptor 400.

11 Reagents and standards

- 11.1 G15: Coulter LS Control G15 (ref 7800370) 15 μ m garnet.
- 11.2 GB500: Coulter LS Control GB500 (ref 7800372) 500 μ m glass beads.
- 11.3 ES63: Engineering sand sieved to produce a size range of 63 μ m < x < 125 μ m.
- 11.4 ES250: Engineering sand sieved to produce a size range of 250 μ m < x < 500 μ m.
- 11.5 1% Acetic acid: Pour 400mL of de-ionized (DI) water into a clean 500-mL volumetric flask. Transfer approximately 5ml of acetic acid into a clean beaker and use a pipette to transfer 5 ml of acetic acid to the flask containing DI water. Dilute to 500ml with DI water. Danger: Corrosive.
- 11.6 30% Hydrogen peroxide (H₂O₂): Pre-made solution that can be purchased from Fisher Scientific, H325-500. Caution: Strong Oxidizer.
- 11.7 3% Sodium hexametaphosphate (NaPO₃n) Dispersant Solution: Weigh 30g of sodium hexametaphosphate in a weighing tray and transfer it to a 1-L volumetric flask. Pour 500ml of de-ionized water into the flask and swirl to dissolve. Dilute to volume with addition DI water. NaPO₃n can be purchased from ELE International, 24-4145 (CAS: 10124-56-8).

12 Quality control

- 12.1 Quality control program: The minimum requirements of the quality control program for this analysis consist of an initial demonstration of laboratory capability and the analysis of background and standard check samples as a

continuing check on performance. The laboratory must maintain performance records that define the quality of the data that are generated.

- 12.2 Initial Accuracy and MDL Determination – See Appendix B to review procedures used to determine initial accuracy, precision, and MDL for these analyses.
- 12.3 Initial Operator Precision and Accuracy – To establish the ability to generate precise and accurate results, the operator shall perform 5 replicates of the grain size standards G15, ES63, and ES250. The mean (\bar{x}), standard deviation (s), and coefficient of variation (CV%) of each particle size category will be calculated for each standard analyzed. The mean values of each particle size category should be within $\pm 10\%$ of the mean values associated with the initial accuracy and precision determination listed in Appendix B to be considered accurate. The CV% of each of the 5 analyses should be within $\pm 20\%$ to be considered precise.
- 12.4 A background measurement provides information about the cleanliness and integrity of the water used for sample dilution and eliminates any interfering signal due to possible light leakage or scattering from dust on lenses or any particulate matter present in the optical path by comparing the current background levels to the background levels set for the instrument during set-up and calibration.
 - a. Tap water, used to dilute each sample, is the only matrix measured for the background determination.
 - b. Tap water is warmed to approximately 50°F within a holding tank and pumped to the ALM to provide consistency that is not normally found from using water from the tap alone. Degassing associated with temperature changes from incoming tap water caused bubbles to form in the sample matrix. These bubbles were not dissociated from particles in the sample, but measured as particles within the sample which decreased accuracy and precision. A holding tank and heaters were added to the system to eliminate the false positives within detector channels. See Appendix C for holding tank information.
 - c. Background is measured between each sample analysis as dictated by the SOM.
 - d. The background measurement serves as a laboratory reagent blank.
 - e. Background flux pattern data is recorded between each sample analysis and is delivered to the QA/QC Coordinator with each project data set. The QA/QC Coordinator randomly chooses a background flux pattern from each carousel, performs calculations, and compares data to the established limits. Background standards are reported as achieved or not achieved with each data report. If background limits are not achieved, the carousel is re-analyzed.

- f. If the background flux pattern is higher in any channel by a factor of two, the contaminant source will be identified, maintenance will occur, corrections will be made, and samples from that carousel will be re-analyzed.
 - g. Contact the laboratory supervisor if faulty background patterns continue to be observed.
- 12.5 The CVS (G15), NS (ES63 and ES250), and LD will be analyzed at the start of the analytical cycle and after every 52 sediment samples or at least twice during a batch analysis. Batch accuracy and precision will be determined by calculating Relative Percent Difference for each particle size classification or value reported (i.e., phi sizes, mean, mode, d10, d50, d90, etc.). The true values listed on the Beckman Coulter Particle Characterization Assay Sheet or the mean values derived from the initial accuracy, precision, and MDL determination will be used to determine accuracy. In addition, all channel data will be reviewed by the QA/QC coordinator for outliers. The RPD for each value should be $\pm 20\%$.
- 12.6 Field Duplicate (FD): Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.
- 12.7 All calibration and quality control information is recorded in the batch information and calibration file associated with the analyses and user.

13 Calibration and standardization

- 13.1 Light scattering is an absolute measurement technology only in the sense that once the experimental setup is correct, calibration or scaling are not necessary in order to obtain the volume (or weight) percentage of each component. Calibration is determined by the optical design, therefore no calibration is required. The instrument measures electrical offsets and aligns the laser beam.
- 13.2 Reference background files are saved in the calibration files folder using the filename format: ZLBYYYY.\$ls. The YYYY are the last four digits of the optical LS 13 320 bench serial number. A reference background file is used to monitor the any changes in the diluent (filtered, room temperature water) over time. A reference background file is not used in this procedure, however, a reference file may be set-up.
- 13.3 The preferences file (.prf) includes options relating to data presentation and output formats. The preference file used in this procedure is: OEWRI_Standard_Preferences.prf. This preference file exports grain size distribution data for each sample as differential volume % for each of the 126 channel listings. Statistical data consisting of mean, median, mode, d10, d50, and d90 is exported for each sample with the preference file as well. Project

specific reference files can be created or edited to fit project specific needs as determined by the project manager and laboratory director.

- 13.4 The Standard Operating Method (SOM) is a program that controls the instrument settings and automatic sampler to ensure consistent analyses and improve precision. The SOM that is used for natural sediment samples is titled OEWRI_Standard_SOM.som and includes preset sample analysis protocol pertaining to run cycle options, Auto Prep Station sample handling, and run settings. Standard templates for SOMs are in the SOP folder and are used by selecting Run and loading the appropriate SOM. The OEWRI Standard SOM measures the LD range from 0.37519 μ m to 2000 μ m and does not include PIDS data. Project specific SOMs can be created or edited to fit project specific needs as determined by the project manager and laboratory director. NEVER MODIFY SOMs. Individual templates with specific SOMs for various media and preferences for data display can be made before analyses and saved specifically for a project. The laboratory manager is the only person authorized to make changes to the SOMs, so these activities need to occur before the day of analyses, plan accordingly. The following settings are used for OEWRI_Standard_SOM.som:

Run Cycle Options: Offsets, alignment, and background measurements will be measured between each sample.

Auto Prep Station sample handling:

- Sample tube is sonicated for 30 seconds on the highest setting (8)
- Fluid is added for 2 seconds during sonication
- Sample tube is flushed and emptied for 3 seconds
- Sample is loaded into the ALM circulation bath and diluted until Standard Obscuration settings are attained.

ALM and Run Cycle Settings:

- Pump speed: This is currently set to 60%. Pump speed should be changed to optimize the project specific samples. You should select a pump speed that is sufficient to suspend the largest diameter particle.
- Run length for particle measurement is 60 seconds and does not include PIDS data.
- Optical model: Default is Fraunhofer.rf780d. After samples are analyzed, other optical models like Mie Scattering may be selected and the data re-computed. See the reference list for more information on studies testing different optical models (e.g., Eshel et al., 2004).

- 13.5 The aqueous liquid module (ALM) is capable of suspending samples in the size range of 0.04 μ m to 2000 μ m. The Polarization Intensity Differential Scattering (PIDS) assembly provides the primary size information for particles in the 0.04 mm to 0.4 mm range.
- 13.6 The instrument will be maintained as defined in the user manual or as QA/QC data dictate.

14 Procedure

- 14.1 Initial sample preparation procedures for natural sediment samples consist of:
- Dry each sample in oven at 60°C. This may take 7 days or more depending on conditions during sample collection.
 - Disaggregate each dry sample with mortar and pestle.
 - Sieve each sample through a 2mm sieve.
- 14.2 Determine whether PIDS data is required for the project. If data from the ultra fine range is desired, sediment samples with require additional sieving and the SOM will have to be edited to include PIDS. If PIDS data is not required, continue with the procedures written in this SOP.

Optimal Sample Mass Determination

- 14.3 Use the following to determine a sample mass that satisfies the “Standard Obscuration” values as recommended by the LS 13 320 software. The appropriate sample mass will minimize the number of dilutions necessary to obtain the correct obscuration values for all channels optimizing the Auto Prep Station. Dilutions provide an acceptable signal-to-noise level in the detector channels, because if too many particles are present, light already scattering from one particle would likely be scattered from another which blurs the light intensity pattern. Representative samples will be chosen from the project, weighed using various masses, manually loaded into the ALM (Aqueous Liquid Module), and analyzed. The *Measure Loading* function, that measures the amount of light scattered out of the beam when particles are introduced into the sample cell, will be used to measure and observe each obscuration value associated with each sample mass. The optimal sample mass for the project will be derived from the obscuration values observed. Follow the procedures below to determine the optimal sample mass for the project.
- Chose samples that represent typical project characteristics. The project manager and laboratory QA/QC coordinator should be consulted.
 - The project manager and laboratory QA/QC coordinator should be consulted to establish the “start mass” and “finish mass” to increase the speed of this determination because optimal sample mass can vary from 100mg to 400mg. Pre-determined masses of each sample will be in 50mg increments to speed processing. Generally, the finer the sample, the less amount of sample that will be required, so samples with more silt or clay will require less sample mass.
 - Place a weighing paper on the balance, tare the balance, and weigh sample. Transfer sample into a bar coded test tube. Continue weighing samples according to predetermined masses and weigh a corresponding duplicate for each predetermined sample mass. For example, if the start mass is 100mg and the finish mass is 200mg, there will be two 100mg, two 150mg, and two 200mg aliquots of **each** representative sample. Transfer each sample into a

bar-coded test tube and record the sample ID and corresponding sample tube bar code on the bench sheet located in Appendix A.

- d. Use the flask with calibrated automatic dispenser labeled “3% Hexameta.soln.” to add 3ml of de-ionized (DI) water to each sample in the tubes. Put on gloves and goggles. Use a pipette to deliver 600 μ l of 30% H₂O₂ to each sample. Use a different pipette to add a 1 drop of 1% acetic acid solution to each sample tube. Monitor the samples after adding the H₂O₂, as some samples may foam aggressively. If samples foam excessively, overwhelming the sample tube, add a small amount of DI water to the sample to dilute the reaction.
- e. Set the Daigger Vortex Genie to the *Touch* setting and set the speed at 4-5. Separately place each tube on the platform and stir each sample for at about 5 seconds.
- f. Place the sample tubes back in the tube rack and let the samples digest for 24 hours.
- g. Set the centrifuge to power 5, insert samples into a centrifuge, and centrifuge each sample for approximately 3 minutes. Decant the clear supernatant liquid using the vacuum pump and attached flask, while retaining all of the sediment in the tube.
- h. Repeat procedures 13.3d through 13.3g.
- i. Observe the sediment. A completely digested sediment sample should be significantly lighter in color than the undigested sediment. Exceptionally organic rich sediments that are very dark brown in color and generally containing > 10% organic matter may require additional digestion treatment. In addition, the sample may require an additional digestion treatment if it effervesces excessively when shaken. Contact the project manager regarding additional treatment procedures.
- j. Use the flask with calibrated automatic dispenser labeled “3% Hexameta.soln.” to add 3 ml of 3% sodium-hexametaphosphate to each sample and stir sample using the Vortex Genie for at about 5 seconds. Place the sample tubes back in the tube rack and allow the samples to soak for approximately 5 minutes.
- k. Sonicate each sample with the independent sonicator (Omni Sonic Ruptor 400) for 1 minute to disperse the sample. To operate the sonifier: turn the power switch to the on position (located on the back of the panel); place the sample tube under the sonicator tip, immerse the tip in the sample; press the Start button and slowly increase the power to 30 (as read on the power output display on the top of the panel). Place the samples tubes back in the tube rack and cover the samples with the bench sheet associated with the samples until they can be manually analyzed.

- I. Manually analyze each sample.
 1. Turn on optical module by selecting **Run** → **Use Optical Module**. Allow optical bench to warm up approximately 15 minutes before beginning analyses.
 2. Check the ALM bath to ensure that dilution water is at the second liquid level sensor. If not, select **Control** → **Fill**.
 3. To analyze samples using the Run Cycle option versus a SOM, click on **Run Cycle**, and then **New Sample**.
 4. Make the following changes to the default settings in the Run Cycle window:
 - i. Deselect the *first* Auto Rinse box, then select Auto Rinse at the bottom of the list.
 - ii. Deselect “include PIDS data”.
 - iii. Make sure the following boxes are checked: Measure offsets, Align, Measure background, Measure Loading, and Start 1 Run.
 - iv. Select “Sonicate during loading” at Power 2.
 - v. Select Options and make sure that the “By Standard Obscuration” is selected under Sample loading Options. Click **OK**.
 - vi. Select **Sample Info**. Fill in the appropriate File Name, Sample ID, and any comments to be saved with the sample. View the filename template in lower left corner to make ensure that the correct sample info was entered. Click OK when finished.
- m. In the Run Cycle window, select **Run Settings** and make necessary changes:
 - i. Set the pump speed to 60. Increase speed if grains remain in the ALM bath.
 - ii. Set **Run Length** for 60 seconds (the default run time).
 - iii. Sonicate during run at Power 2.
 - iv. The number of runs is 1.
 - v. Check boxes for Compute Sizes during run and Save File.
 - vi. Nothing should be selected under “Before first run” or “Between Runs.”
 - vii. Select **Model** and click on Fraunhofer.rf780d.
 - viii. Select **Folder** and change the folder directory where the sample data will be saved if necessary.
 - viii. Click OK when completing all of these steps within the Run Cycle window and proofing changes made.
- n. In the Run Cycle window, click **Start** to initiate the instrument to measure offsets, auto align, and measure background. Observe background results. If the background flux pattern is higher in any channel by a factor of two, identify the contaminant source, make corrections, and re-analyze the sample.
- o. When the screen reads “Obscuration 0%: Low, Add Sample” add the first sample mass to the ALM bath.

- p. Monitor the laser diffraction obscuration values at the top of the Measure Loading window.
- i. If the screen reads “Obscuration XX%: Low, Add Sample”, the obscuration is unacceptable and the sample concentration is too low, skip to step “q.” to flush the system and allow the analysis of a larger pre-measured mass from the set.
 - ii. If obscuration is “High”, dilution is necessary, select **Control → Dilute Once**. Allow the sample obscuration values to stabilize and monitor the new obscuration values. If the values are still not acceptable, dilute once again. Keep track of how many times the sample is diluted. Do not dilute more than 3 times, skip to step “q.” to flush the system and allow the analysis of a smaller pre-measured mass from the set.
 - iii. If obscuration is “OK” the initial obscuration is acceptable as is the sample mass associated with the analysis. Note this mass and continue to step “q.”
- q. Select **Start Analysis** which is located in the lower left corner of the screen. This will measure the sample regardless of obscuration and flush the system to allow the analysis of the next pre-measured mass from the set.
- r. Continue to analyze each sample mass by repeating step “p” or until the optimal sample mass is determined.
- s. Operators will inform the QA/QC coordinator upon completion of the sample mass analyses.

QA/QC Preparation

14.4 The CVS (G15), NS (ES63 and ES250), and LD will be analyzed at the start of the analytical cycle and after every 52 sediment samples or at least twice during a batch analysis. Each carousel holds a maximum of 30 samples. The CVS (G15), NS (ES63 and ES250), and LD will occupy the first four slots of the first project carousel and will occupy slots 26-30 of the second carousel. Choose a laboratory duplicate randomly per carousel from samples being analyzed from that carousel. Use Table 2 to prepare the CVS, NS, and LD.

Table 1. QA/QC Preparation

Source	G15	ES63	ES250	LD
Mass (mg)	150	2500	6000	Optimal mass determined in Section 13.3

Place a weighing paper on the balance, tare the balance, and weigh each standard or laboratory duplicate. Transfer each mass into a bar-coded test tube and record the sample ID and corresponding sample tube bar code in a project data book. **The CVS's (G15) and NS (ES63 and ES250) do not require any pretreatment.** Load these tubes into the appropriate slots in the carousel.

Sample Preparation

- 14.5 After determining the optimal sample mass in Section 13.3, take each sample through the following procedures.
- a. Place a weighing paper on the balance, tare the balance, and weigh sample. Transfer each sample into a bar-coded test tube and record the sample ID and corresponding sample tube bar code on a bench sheet.
 - b. Use a flask with a calibrated automatic dispenser and labeled "DI Water" to add 3ml of de-ionized (DI) water to each sample in the tubes. Put on gloves and goggles. Use a pipette to deliver 600 μ l of 30% H₂O₂ to each sample. Use a different pipette to add a 1 drop of 1% acetic acid solution to each sample tube. Monitor the samples after adding the H₂O₂, as some samples may foam aggressively. If samples foam excessively, overwhelming the sample tube, add a small amount of DI water to the sample to dilute the reaction.
 - c. Set the Daigger Vortex Genie to the *Touch* setting and set the speed at 4-5. Separately place each tube on the platform and stir each sample for at about 5 seconds.
 - d. Place the sample tubes back in the tube rack and let the samples digest for 24 hours.
 - e. Set the centrifuge to power 5, insert samples into a centrifuge, and centrifuge each sample for approximately 3 minutes. Decant the clear supernatant liquid into a 250ml beaker while retaining all of the sediment in the tube.
 - g. Observe the sediment. A completely digested sediment sample should be significantly lighter in color than the undigested sediment. Exceptionally organic rich sediments that are very dark brown in color and generally contain > 10% organic matter may require additional digestion treatment. In addition, the sample may require an additional digestion treatment if it effervesces excessively when shaken. Contact the project manager regarding additional treatment procedures.
 - h. Use the flask with calibrated automatic dispenser and labeled "3% Hexameta.soln." to add 3 ml of 3% sodium-hexametaphosphate to each sample and stir sample using the Vortex Genie for at about 5 seconds. Place the sample tubes back in the tube rack and allow the samples to settle for approximately 5 minutes.

- i. Sonicate each sample with the independent sonicator (Omni Sonic Ruptor 400) for 1 minute to disperse the sample. To operate the sonifier: turn the power switch to the on position (located on the back of the panel); place the sample tube under the sonicator tip, immerse the tip in the sample; press the Start button and slowly increase the power to 30 as read on the power output display on the top of the panel. Place the samples tubes back in the tube rack and cover the samples with the bench sheet associated with the samples until they can be manually analyzed.

Pre-analysis Procedures

14.6 Access the software and load the carousel following the procedures below.

- a. Turn on the power strip located on the wall above the pump. The pump and water heaters are plugged into this power strip. See Appendix C for water tank information. Observe the water temperature. It is recommended that water temperature be 20°C (68°F) or above for sample analyses. Record the dilution water temperature in the project notebook.
- a. Record ambient laboratory conditions such as temperature and humidity in the project notebook.
- b. Turn on computer and log on with user name and password.
- c. Click LS 13320 desktop icon. Click **OK** to acknowledge the copyright and log on with user name and password to access the software.

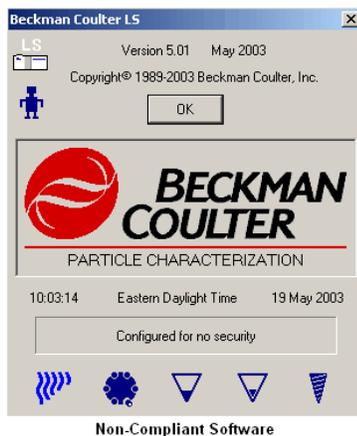


Figure 2. LS 13320 logon screen.

- d. Click **Run** → **Use Optical Module**.

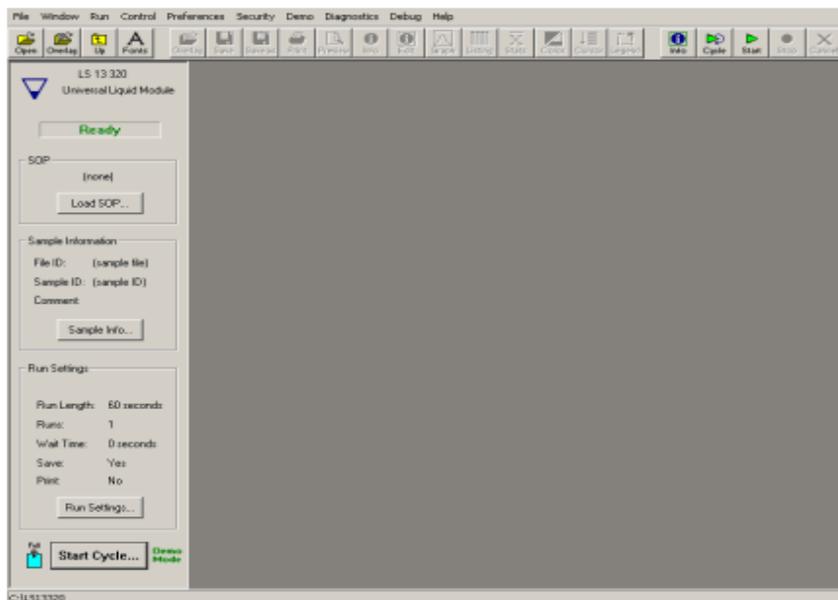


Figure 3. LS 13320 Optical Module screen.

- e. **Click Control → Pump On** to turn the ALM pump on. Make sure that the ALM bath is free of debris and water is to the fill line. Rinse the bath to remove particles by selecting **Control → Rinse** and allow the bath to rinse until it appears clean. Click **Cancel** on the ALM rinse window to turn the rinse cycle off. The bath will automatically fill to the correct water level when the rinse is turned off.
- f. Advance the Auto Prep Station (APS) carousel to the home position, by selecting **Control → Carousel to Home**. The carousel will automatically advance Slot 1 to the home position which is just before the APS sonicating device.
- g. Load sample tubes into carousel slots, according to the table below :

Table 2. Sample Loading Order

Carousel #1		
Sample Type	Carousel Slot No.	SOM File Used
G15 Standard	1	G15_Garnet_SOM_alm_ap
ES63 Standard	2	E63_Sand_SOM_alm_ap
ES250 Standard	3	ES250_SOM_alm_ap
Lab Duplicate	4	OEWRI_Standard_SOM
Natural Sediments	5-30	
Carousel #2		
Sample Type	Carousel Slot No.	SOM File Used
Natural Sediments	1-26	OEWRI_Standard_SOM
G15 Standard	27	G15_Garnet_SOM_alm_ap
ES63 Standard	28	E63_Sand_SOM_alm_ap
ES250 Standard	29	ES250_SOM_alm_ap
Lab Duplicates	30	OEWRI_Standard_SOM

- h. If the project requires additional carousels, odd numbered carousels should mirror the sample loading order of Carousel #1 and even numbered carousels should mirror the sample loading order of Carousel #2. All project analyses should end with the analysis of each standard and a laboratory duplicate from a sample within the carousel.
- i. Tip each tube toward the center of the carousel to facilitate carousel movement.
- j. Continue with procedures in Section 14.7 after the instrument has been powered on for at least 15-minutes.

Sample Analysis

14.7 Select **Run** → **Run Auto-Prep Station** from the toolbar. A window entitled “Run the Auto-Prep Station – By slot number” like the one below should be visible.

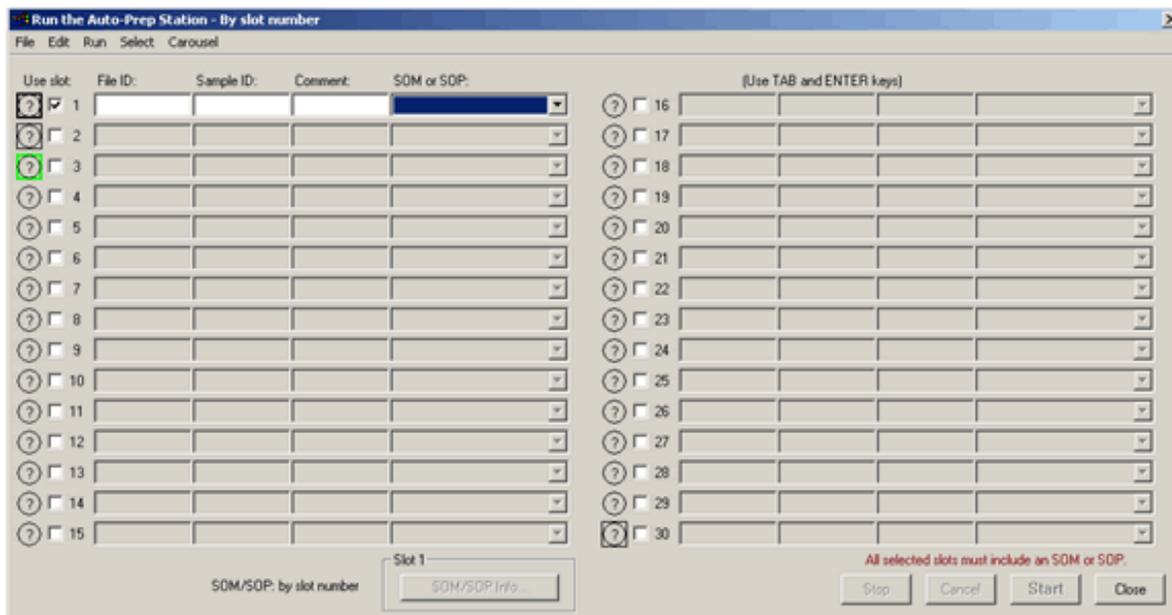


Figure 4. “Run the Auto-Prep Station – By slot number” screen.

- 14.8 Activate Slot 1 that contains the G15 Lab Standard by clicking the box next to the slot number. Type the File ID, Sample ID, and any necessary comments into the row that corresponds with the carousel slot chosen. Choose the appropriate SOM from the drop down list in the column labeled “SOM or SOP”. Appropriate SOMs are listed in Table 3 above.
- 14.9 Activate Slot 2 that contains the ES63 Natural Standard by clicking the box next to the slot number. Type the File ID and Sample ID information into the column that corresponds with the carousel slot chosen. Add any additional information to the Comments box. Choose the appropriate SOM from the drop down list in the column labeled “SOM or SOP”. Appropriate SOMs are listed in Table 3 above.
- 14.10 Activate Slot 3 that contains the ES250 Natural Standard by clicking the box next to the slot number. Type the File ID and Sample ID information into the column that corresponds with the carousel slot chosen. Add any additional information to the Comments box. Choose the appropriate SOM from the drop down list in the column labeled “SOM or SOP”. Appropriate SOMs are listed in Table 3 above.
- 14.11 Continue to activate each of slots 4 through 30 (or the appropriate ending slot number) by clicking the box next to the slot number and typing the File ID’s and sediment sample IDs. Choose the appropriate SOM from the drop down list in the column labeled “SOM or SOP”. Appropriate SOMs are listed in Table 3 above.
- 14.12 Review all information in the “Run the Auto-Prep Station – By slot number” window to ensure that all sample IDs correspond to the correct tube and slot number.

- 14.13 Click Start to begin analysis. The instrument will measure offsets, align the laser and detector, and measure background. The screens will look similar to those below.

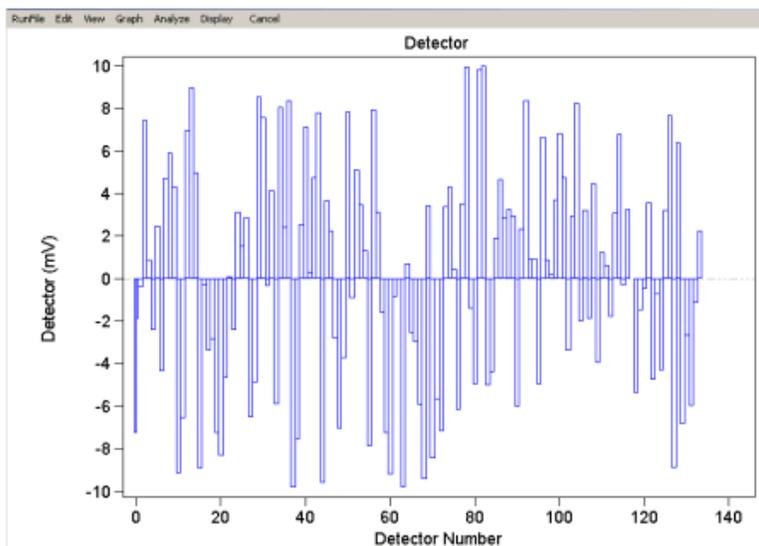


Figure 5. Measuring offsets screen.

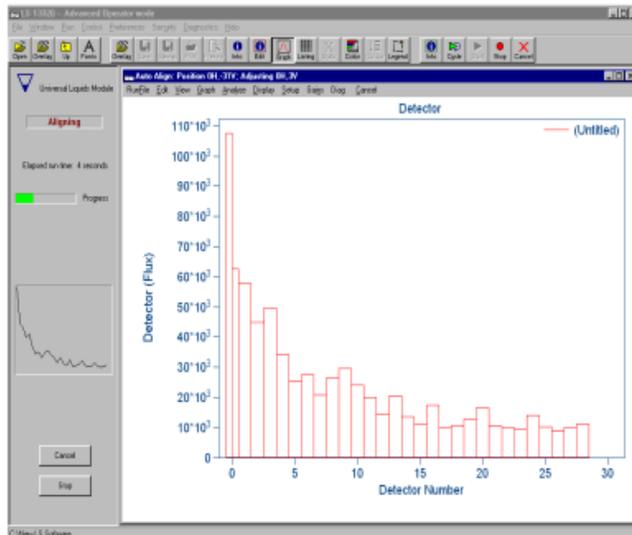


Figure 6. Auto align screen.

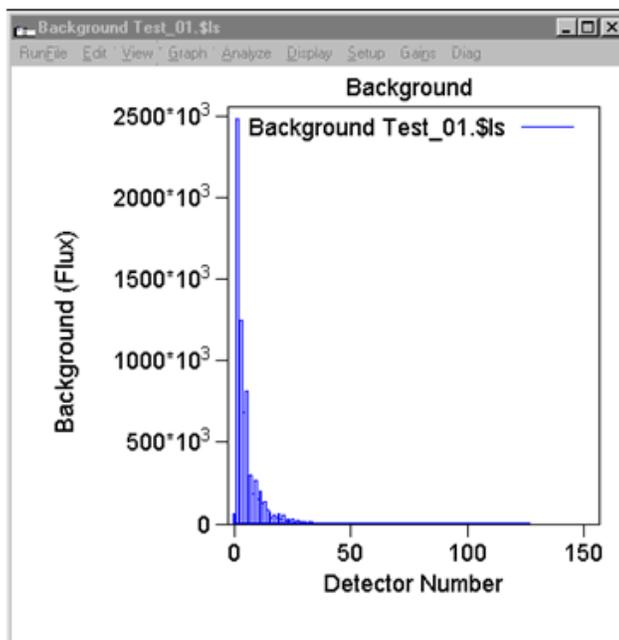


Figure 7. Normal background screen.

- 14.14 Observe the background intensity flux patterns test box. The graph should mirror the one above. Contact the laboratory supervisor if excessively high or low background patterns are observed. Background flux patterns for each carousel should be reviewed before many additional samples are analyzed to decrease the number of samples that may have to be re-analyzed due to unacceptable variations in the background flux patterns. To check the graph of the background flux pattern on a sample run click View, then Intensity, then Graph followed by Background (Flux).
- 14.15 The operator can leave the instrument after all samples are loaded and background measurements are determined to be within limits, however, the instrument should be monitored periodically during sample analysis.
- 14.16 As sample measurements are completed, the carousel may be filled and slots re-selected while the remaining samples are analyzed. As the sample analysis is completed, the sample slot information becomes gray. To refill the slot with additional samples, reactivate the Slot number by checking the box next to the slot number and filling in the appropriate information. Be sure and insert the correct sample tube for the sample information entered and change SOM information if necessary.
- 14.17 Refer to Section 15.1 for data retrieval procedures.
- 14.18 Instrument Shut Down
- At the end of the analyses click **Control** → **Pump off**.

- b. Flip the switch to off on the power strip located on the wall above the pump to turn off the holding tank water pump and water heaters.
- c. Turn the optical module off by exiting the software.
- d. Log off and turn the computer off.

14.19 Area Clean Up

- a. Use a damp sponge to wipe the carousel and area around the ALM clean.
- b. Remove sample tubes from the ALM bin and wipe out the bin.
- c. Rinse used sample tubes with DI water and place them upside down in a rack to dry.
- d. Close the ball valve between the water filter and copper water line by moving the lever to the closed position.
- e. Remove sample bags and other personal items from the area.

15 Data acquisition, calculations, and reporting

15.1 Follow the procedures below to acquire particle size data. Data is stored as “.\$ls” files that the LS 13 320 software uses for data presentation.

- a. Load the preference file: OEWRI_Standard_Preferences.prf. This Preference file exports the following data for each sample:
 - i. Sample information in the formats illustrated below: analysis time and date, file name, file ID, Sample ID, operator, bar code, and comments.

Table 3. Sample Information Format of Preferences File

LS	9:41 8 Nov 2007
File name:	Dig Test_2Dig-H2_2007-11-07_13-19_01.\$ls
File ID:	Dig Test
Sample ID:	2Dig-H2
Operator:	Gwenda J. Schlomer
Bar code:	
Comment 1:	
Comment 2:	

- ii. Channel list with corresponding differential % volume detected with particle analysis in that channel:

Table 4. Channel Information Format of Preferences File

	Dig Test	Dig Test	Dig Test
Channel	_2Dig-H2	_2Dig-L1	_2Dig-L2
Diameter	_2007-11	_2007-11	_2007-11
(Center)	-07_16-45	-07_16-02	-07_16-10
um	_07.\$ls	_02.\$ls	_03.\$ls
	Diff. Volume	Diff. Volume	Diff. Volume
0.393	1.22E-06	0.002138	0.00209
0.432	1.98E-05	0.00263	0.002587
0.474	0.000127	0.003904	0.003872
0.520	0.000456	0.007353	0.007348
0.571	0.000961	0.01553	0.015503
0.627	0.001736	0.030317	0.030094
...
1314	0	0	0
1443	0	0	0
1584	0	0	0
1739	0	0	0
1909	0	0	0

- iii. Statistics for each sample include sample mean, mode, d10, d50, and d90.
- b. To view data in the LS 13 320 software, click **Open** and navigate to the correct directory. Select the file to view and click **Open**.
- c. To export data for a single sample, click **Open** and navigate to the correct directory. Select the file to export and click **Open**.
- d. To export or view more than one file at the same time, select **Overlay** and navigate to the appropriate file folder. Files within the folder are listed in the center column of the Overlay window. Select the files to view and click **Add**. The selected files can be viewed in the column on the right. A maximum of 20 files may be viewed at one time. Click **OK** to view the files.

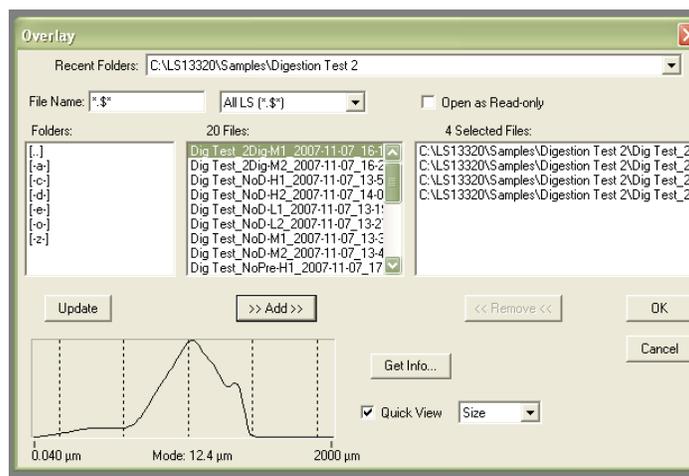


Figure 8. Overlay screen.

- e. To export multiple files at one time as an Excel (.xls) file select **Overlay** and navigate to the appropriate file folder. Files within the folder are listed in the center column of the Overlay window. Select the files to view and click **Add**. The selected files can be viewed in the column on the right. A maximum of 20 files may be viewed at one time. Make sure that the options illustrated below are checked in the overlay window. Click **OK** to view the files. Click **RunFile** → **Export** to open the Export Data window.

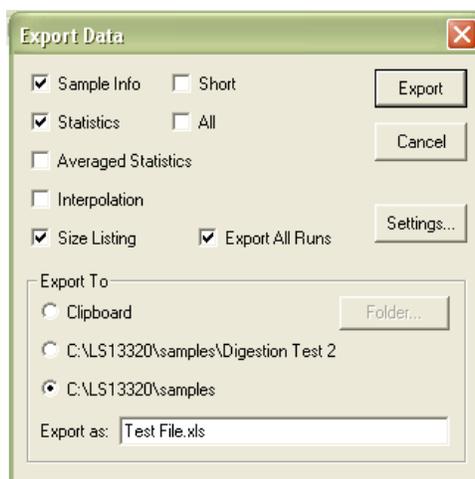


Figure 9. Export Data screen.

In the Export Data window, select the following boxes: Sample Info, Statistics, Size Listing, and Export All Runs. Choose the appropriate directory and file option. To use a directory other than the default directory, select the middle option under "Export To", then select **Folder** and navigate to the appropriate file folder. Click **OK**. In the "Export As" text box, type in an appropriate file name for the exported data file. Make sure to leave the Excel file extension (.xls) at the end of the file name. Click **Export**. Repeat the overlay and export procedures until all the samples for the project have been exported

into an Excel file. Copy the exported folder to a removable flash drive for further processing in Excel.

- 15.2 Follow the procedures below to export background data.
- a. Load the preference file: Background_Intensity_Listing.prf. This Preference file exports the background intensity flux count for each channel and the corresponding detector number for each sample selected:

Table 5. Background Intensity Listing of Preferences File

Detector Number	BDWetlands BD1AO_	BDWetlands BD1BI_	BDWetlands BD1BO_
	_2008-03	_2008-03	_2008-03
	-18_13-30	-18_13-38	-18_13-46
	_0...	_0...	_0...
	Background	Background	Background
0	38400	31560.5	31416.4
0.5	45114.9	38926.7	39501.1
1	631259	616961	623469
2	1.34E+06	1.27E+06	1.26E+06
3	510794	492858	488354
4	370289	341680	331661
...
124	10.5355	10.1875	10.5624
125	9.99587	9.68284	10.0814
126	9.6515	9.36997	9.77349
127	0	0	0

- b. To view and export the background intensity flux for associated with samples previously exported, select **Overlay**, add the appropriate files to the file list, and click **OK** (to view the files). In the **Overlay** window, select **Graph → Background (flux)**, then **View → Listing**. Channels 0 to 127 should be listed.
- c. In the **Overlay** window, click **RunFile → Export**.

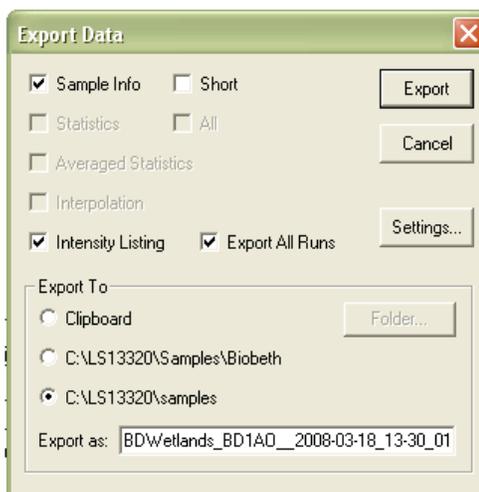


Figure 10. Export Background Data screen.

In the Export Data window, select the following boxes: Sample Info, Intensity Listing, and Export All Runs as illustrated above. Choose the appropriate directory and file option. To use a directory other than the default directory, select the middle option under “Export To”, then select **Folder** and navigate to the appropriate file folder. Click **OK**. In the “Export As” text box, type in an appropriate file name for the exported data file. Make sure to leave the Excel file extension (.xls) at the end of the file name. Click **Export**. Repeat the overlay and export procedures until all the samples for the project have been exported into an Excel file. Copy the exported folder to a removable flash drive for further processing in Excel.

15.3 Reporting results: Results should be reported to 0.01 %.

15.4 Relative percent difference (RPD):
$$\left[\frac{(A-B)}{\text{AVERAGE}(A,B)} \right] 100$$

A = original sample concentration
 B = duplicate sample concentration

15.5 Standard Deviation: The evaluation of MDL and precision require calculation of standard deviation. Standard deviations should be calculated as in equation 2.

$$s = \left\{ \frac{\sum x^2 - [(\sum x)^2/n]}{n - 1} \right\}^{1/2}$$

Where: n = number of samples,
 x = concentration in each sample.

Note: This is the sample standard deviation calculated by the STDEV function in Microsoft Excel.

- 15.6 Coefficient of Variation (Cv%): Accuracy can be calculated by determining the coefficient of variation which is the standard deviation of the multiple sample measurements divided by the mean of those measurements.

$$Cv\% = (s/\bar{x})100$$

16 Computer hardware and software

16.1 Microsoft Word: this document is prepared using Word.

16.2 The Word document file name for this SOP is: Partical Sizer R01.doc

17 Method performance

17.1 Method performance data for SOMs are available through the LS 13 320 software. The aqueous liquid module (ALM) is capable of suspending samples in the size range of 0.04 μm to 2000 μm . The Polarization Intensity Differential Scattering (PIDS) assembly provides the primary size information for particles in the 0.04 mm to 0.4 mm range. The PIDS assembly also enhances the resolution of the particle size distributions up to 0.8 mm. Repeatability is 1% about mean size.

17.2 The desired performance criteria for these analyses are:

- a. There are three background flux intensity limits established by the manufacturer and are listed below:
 - i. The flux counts within Channels 1 and 2 must be $<4.0 \times 10^6$.
 - ii. The flux counts for the sum of Channels 10 through 40 must be $<2.0 \times 10^6$.
 - iii. The Channel between 95 and 126 that has the highest flux counts cannot exceed 30.

Background flux pattern data is recorded between each sample analysis and is delivered to the QA/QC Coordinator with each project data set. The QA/QC Coordinator randomly chooses a background flux pattern from each carousel, performs calculations, and compares data to the established limits. Background standards are reported as achieved or not achieved with each data report. If background limits are not achieved, the carousel is re-analyzed.

- b. Precision: $\pm 20\%$, precision data will be reported as relative percent difference and project managers will determine what data is used.
- c. Accuracy: $\pm 20\%$, accuracy data will be reported as relative percent difference and project managers will determine what data is used.

- d. Minimum Quantification Interval: 0.01%.

18 Pollution prevention

All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU College of Natural and Applied Sciences. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

19 Data assessment and acceptable criteria for quality control measures

- 19.1 The analyst should review all data for correctness.
- 19.2 Relative percent difference (RPD) will be calculated for pairs of duplicate analyses to determine precision. Two laboratory duplicates (LD) will be randomly selected from each set of 52 samples and analyzed. Precision data will be reported as relative percent difference and project managers will determine what data is used. The desired precision is $\pm 20\%$.
- 19.3 The CVS (G15) and NS (ES63 and ES250) will be analyzed at the start of the analytical cycle and after every 52 sediment samples or at least twice during a batch analysis. Accuracy will be determined by calculating Relative Percent Difference for the true values listed on the Beckman Coulter Particle Characterization Assay Sheet. All channels will be reviewed by the QA/QC coordinator for accuracy. The RPD should be $\pm 20\%$.
- 19.4 The background flux intensity limits are as follows:
- The flux counts within Channels 1 and 2 must be $<4.0 \times 10^6$.
 - The flux counts for the sum of Channels 10 through 40 must be $<2.0 \times 10^6$.
 - The Channel between 95 and 126 that has the highest flux counts cannot exceed 30.
- 19.5 The background flux pattern cannot vary in any channel by a factor of 2 or higher.
- 19.6 The completed Excel spreadsheet is reviewed by the analyst's supervisor or the OEWR QA officer.

20 Corrective actions for out-of-control or unacceptable data

- 1201 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.
- 20.2 The results for background, precision, and accuracy are compared to acceptable values for this analysis. If any results fail to meet criteria, the samples associated with the failed criteria will be reanalyzed.
- 20.3 The instrument may require trouble shooting techniques if the data are unacceptable. All instrument maintenance will be performed as outlined in

manual and by the laboratory manager or designated technician. Refer to Appendix D for common troubleshooting techniques.

21 Waste management

The wastes generated in this method are not hazardous.

22 References

Blott, S.J., Croft, D.J., Pye, K., Saye, S.E., and Wilson, H.E., 2004. *Particle size analysis by laser diffraction*. Pye, K. and Crofts, J.D. (eds). Forensic Geoscience: Principles, Techniques, and Applications. Geological Society, London, Special Publications, 232: 63-73.

Eshel, G., Levy, G.J., Mingelgrin, U., and Singer, M.J., 2004. *Critical evaluation of the use of laser diffraction for particle-size distribution analysis*. Soil Science Society of America Journal, 68: 736-734.

Loizeau, J.L., Arbouille, D., Santiago, S., and Vernet, J.P., 1994. *Evaluation of a wide range laser diffraction grain size analyzer for use with sediments*. Sedimentology 41: 353-361.

LS 13 320 Laser Diffraction Particle Size Analyzer Instrument Manual © 2003 Beckman Coulter, Inc., 11800 SW 147th Ave., Miami, FL 33196

23 Tables, diagrams and flowcharts

23.1 The diagrams for this method are included in this SOP. Tables and flowcharts can be found in the instrument manual.

23.2 The laboratory bench sheet is attached. The analyst should make a copy of this form for each rack of samples that will be prepared for analysis.

24 Contacts for Additional Instrument Support

24.1 Dana Leech, Senior Customer Support Engineer (Beckman Coulter): (305) 606-5694, Dana.leech@Coulter.com

24.2 Mervin Yaremko, Senior Customer Tech Support (Beckman Coulter call center): (800) 742-7694 Mervin.Yaremko@Coulter.com

APPENDIX B – Initial Accuracy and MDL Determination

Two Calibration Verification Standards (CVS) and two Natural Standard (NS) were analyzed 10 times and results were used to determine initial accuracy and the Method Detection Limit (MDL) in the targeted size ranges associated with the standard. CVS used for this procedure are provided by the instrument manufacturer (Beckman Coulter) and consist of two size fractions: 15 µm garnet (G15) and 500 µm glass beads (GB500). The NS has a known particle size distribution and has composition similar to environmental samples being analyzed. OEWRI projects use the ES63 and ES250 which is an engineering sand sieved to produce a size range of 63µm < x < 125µm and 250µm < x < 500µm.

Data from each channel was combined to produce the following size categories based on Phi sizes commonly used to characterize class limits (National Research Council):

Table 6. Size Categories (National Research Council)

Size Category Name	Size Category Abbreviation	Size Range (µm)
Very Coarse Sand	VCS	2000 - 1000
Coarse Sand	CS	1000 - 500
Medium Sand	MS	500 - 250
Fine Sand	FS	250 - 125
Very Fine Sand	VFS	125 - 62.5
Course Silt	CSi	62.5 - 31.3
Medium Silt	MSi	31.3 - 15.6
Fine Silt	FSi	15.6 - 7.8
Very Fine Silt	VFSi	7.8 - 3.9
Clay	Clay	3.9 - 0.4
Very Fine Clay		<0.4

The CVS G15 provides accuracy data for the MSi and FSi size categories. The NS ES63 provides accuracy data for the FS and VFS size categories. The NS ES250 provides accuracy data for the MS size category. The CVS GB500 provides accuracy data for the CS size category.

Accuracy of these initial analyses was determined by calculating the Relative Percent Difference (RPD) using data from the Beckman-Coulter Control Assay Sheets associated with the CVS and previous accuracy analyses of the NS. Beckman-Coulter only provides the mean value of the data set on the assay sheet for the G15 standard and only provides the d10, d50, and d90 of the data set for the GB500 standard. The acceptable RPD is ±10% and all standards passed QA/QC for accuracy.

Table 7. Accuracy Determination

Standard	True Values	MDL Analysis Values (n=10)	RPD
G15	Mean = 15.14	Mean = 14.29	5.81
ES63	FS = 55.37	FS = 50.89	8.43
	VFS = 42.35	VFS = 45.98	-8.21
GB500	d10 = 502	d10 = 505.1	0.62
	d50 = 563	d50 = 568.7	1.01
	d90 = 632	d90 = 639.9	1.24

Precision of these initial analyses was determined by calculating the Coefficient of Variation (CV%) between size category data from each of the 10 analyses for each of the standards. The

acceptable RPD is $\pm 10\%$ and all standards passed QA/QC for precision relative to the target size categories.

Table 8. Precision Determination

Standard	Size Category	CV%
G15	MSi	0.82
	FSi	0.57
ES63	FS	6.05
	VFS	5.68
ES250	MS	2.10
GB500	CS	0.42

The initial accuracy determination established standard size category mean values for each standard within the target size category and those means are listed below. The NS ES250 was analyzed 10 times to provide accuracy data for the MS size category and will be used for regular accuracy determinations due to cost associated with the GB500 laboratory standard.

Table 9. Mean Values for Regular Accuracy Determinations

Standard	Size Category	Mean Value (Differential Volume Percent)
G15	MSi	43.03
	FSi	36.26
ES63	FS	50.89
	VFS	45.98
ES250	MS	66.22
GB500	CS	95.85

These mean values will be used to compare data from each analysis of each standard and determine accuracy of each batch of samples as described within this SOP.

The typical procedures used to determine the method detection limit are not applicable for analyses using the LS 13 320. Measure loading and offset functions used for LS 13 320 analyses do not allow the measurement of a laboratory blank. Prescribed obscuration percentages are specified in the Standard Operating Method (SOM) and samples are diluted until the specified obscuration percentage is obtained to provide an acceptable signal-to-noise level in the detector channels. Blanks are not recognized in the software and obscuration values set for sample analysis cannot be obtained if no sample is present during measurement. Therefore background flux patterns are used in place of actually analyzing a laboratory blank. The background flux pattern provides information about the cleanliness and integrity of the tap water used for sample dilution. There are three background flux intensity limits established by the manufacturer and are listed below:

- a. The flux counts within Channels 1 and 2 must be $< 4.0 \times 10^6$.
- b. The flux counts for the sum of Channels 10 through 40 must be $< 2.0 \times 10^6$.
- c. The Channel between 95 and 126 that has the highest flux counts cannot exceed 30.

Background flux pattern data is recorded between each sample analysis and is delivered to the QA/QC Coordinator with each project data set. The QA/QC Coordinator randomly chooses a background flux pattern from each carousel, performs calculations, and compares data to the

established limits. Background standards are reported as achieved or not achieved with each data report. If background limits are not achieved, the carousel is re-analyzed.

APPENDIX C - Water Tank Assembly for Beckman Coulter LS 13 320



Photo 1. Water Tank Assembly for Beckman Coulter LS 13 320.

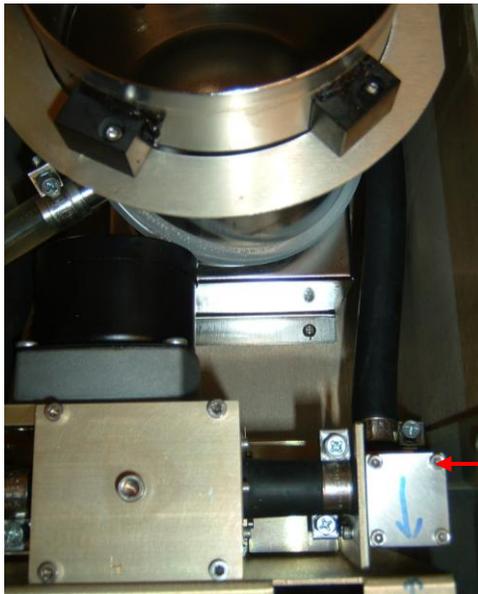
PARTS:

1. Water Tank: 35-gallon, Roto Mold
2. Water Filter Cartridges: CUNO Ultra Block Filter Model GRUBB5N- 5 μ m rating (*Grainger* product #5PT91)
3. Water Filter Housing: USOM-38-S Point-of-Use Water Filter (from *Grainger*)
4. Water Pump: Iwaki Magnetic Drive Pump Model MD-70RLZT
5. Float Valve: Nylon Space-Saving Float Valve (www.mcmaster-carr.com)
6. Heaters: 2- 200Watt Submersible Aquarium Heaters
7. Ball Valves: $\frac{3}{4}$ inch (NPT), between tank and pump; $\frac{1}{2}$ inch (NPT), between pump and instrument and between instrument and tank.
8. Bleeder Valves: $\frac{1}{2}$ inch size.

APPENDIX D

Troubleshooting

- 1. “Backgrounds too negative in 1 channels. (99)”:** Sample material may be collecting in the instrument causing high diluent background values (i.e., the background flux patterns measured during the Measure Background function detected residual material in the water prior to the sample being loaded). This problem usually occurs if the pump speed is too low or sample size is too large. Adjust sample size and/or pump speeds as appropriate if this problem occurs frequently. A thorough cleaning of the instrument will generally remediate the problem. To clean the instrument:
 - Mix up 10% cleaning solution (Micro Cleaner): add 10ml of cleaner to 1-liter volumetric flask; dilute to volume.
 - Select Control → Rinse; allow instrument to rinse for at least 5 minutes.
 - Select Control → Open Drain; fill the instrument (in the ALM) with the cleaning solution.
 - Allow the cleaning solution to circulate in the instrument for at least 1-hour.
 - Drain the instrument and rinse for about 5-min, or until the diluent appears clean (suds-free).
- 2. Loud beeping noise (liquid level sensor), sometimes accompanied by an error message, “The vessel is too full, please drain some fluid.”:** The overflow liquid level sensors in the ALM bath detect fluid on the sensor therefore the instrument alerts the user that the vessel is overflowing. Check the sensors for water droplets and wipe them clean. The beeping should stop.
- 3. Unable to drain instrument (after selecting Control → Open Drain):** Sample material, especially if running a large quantity of fine material, tends to accumulate around a brass ball located at the 90° turn of the inlet hose in the lower right corner of the ALM. To fix this problem, remove the cover of the ALM, and find the metal plate that houses the brass ball (see photo below). Carefully detach the metal plate with an allen wrench (size 5/64 in or 2mm) then using tweezers, lift the metal ball and quickly check to make sure there is no residue on the ball. Be careful not to lift the ball out of place for very long as the water in the line is under pressure and removing the ball will cause water to flow from the line into the ALM. Most of the time, the ball just needs to be briefly lifted or adjusted instead of cleaned.



Remove these bolts using an allen wrench (5/64 in). Reattach as arrows indicate.

Photo 2. Inside the ALM: illustrates a portion of the ALM bath and the 90° turn of the inlet hose in the lower right corner of the ALM.